# Chapter 1

# The Burton Assay for DNA


## *Jaap H. Waterborg and Harry R. Matthews*

*Department of Biological Chemistry, University of California School of Medicine, Davis, California*


## Introduction

The Burton assay for DNA is a colorimetric procedure for measuring the deoxyribose moiety of DNA. It is reasonably specific for deoxyribose, although very high concentrations of ribose (from RNA) or sucrose must be avoided. The method can be used on relatively crude extracts and in other circumstances where direct measurement of ultraviolet absorbance of denatured DNA is not practical. The assay has been widely used.

## Materials

1. Diphenylamine reagent: Dissolve 1.5 g of diphenylamine in 100 mL glacial acetic acid. Add 1.5 mL of concentrated (98–100%) $H_2SO_4$ and mix well. Store this reagent in the dark. Just before use, add 0.5 mL of acetaldehyde stock solution.

2. Acetaldehyde stock: 2 mL acetaldehyde in 100 mL distilled water. Store at 4°C where it is stable for a few months.
3. 1*N* perchloric acid (PCA).
4. *Standards*: Dilute a DNA stock solution with distilled water as follows:

| DNA stock, μL | 0 | 10 | 20 | 50 | 100 | 200 |
|---|---|---|---|---|---|---|
| Water, mL | 1.0 | 0.990 | 0.980 | 0.950 | 0.900 | 0.800 |
| DNA concentration, μg/mL | 0 | 10 | 20 | 50 | 100 | 200 |

5. DNA stock: 1 mg/mL in distilled water. Store frozen at −20°C where it is stable for a few months.

## Method

1. Extract the sample as required (*see* Notes).
2. Add 0.5 mL of 1*N* PCA to 0.5 mL of sample or standard. Hydrolyze for 70 min at 70°C.
3. Cool the hydrolyzed samples on ice for 5 min. Centrifuge (1500*g*; 5 min; 4°C) and decant the supernatants into marked tubes.
4. Add 1 mL of 0.5*N* PCA to each pellet, vortex, repeat step 3, and carry the combined supernatants forward to step 5. (This step is optional; *see* Note 3)
5. Add 2 vol. of diphenylamine reagent to 1 vol of the supernatants (0.5*N* PCA hydrolyzates from step 3). Mix and incubate at 30°C for 18 hr.
6. Read the absorbance at both 595 and 650 nm, using the 0 μg/mL standard as a blank.
7. Plot a standard curve of absorbance at 595 nm minus absorbance at 650 nm as a function of initial DNA concentration and then use the curve to read off unknown DNA concentrations.

## Notes

1. Extraction conditions may have to be optimized for particular applications. The following procedures are routinely used in our laboratory.

(a) This extraction is required if the sample contains mercaptoethanol, dithiothreitol, or other interfering low molecular weight substances. It is also required as a preliminary if extraction (b), which is for whole cells or organelles that contain lipids, is to be used. Add 1 vol. of 0.2*N* PCA in 50% ethanol:50% distilled water and mix by vortexing. Cool on ice for 15 min and then centrifuge (5 min, 1500*g*, 4°C). Discard the supernatant.

(b) To the pellet add 1 mL of ethanol–ether (3:1, v/v). Incubate for 10 min at 70°C. Centrifuge (5 min, 1500*g*) and discard the supernatant. To the pellet add 1 mL of ethanol (96%), vortex and centrifuge (5 min, 1500*g*). Discard the supernatant.

2. If the sample is a pellet, at step 2 add 1 mL of 0.5*N* PCA to the pellet and proceed with the hydrolysis at 70°C. If the sample is too dilute ( $< 10$ μg/mL) and is available in a volume larger than 0.5 mL, then it may be concentrated by precipitation, as described in Note 1, extraction (a), or by precipitation with 0.5*N* PCA.
3. Step 4 is optional. It provides a more quantitative recovery of nucleic acid in the supernatant, but reduces the sensitivity of the overall assay.
4. The diphenylamine reagent is not water soluble. Rinse out glassware with ethanol before washing in water. Take care to use a dry spectrophotometer cuvet and clean it with ethanol.
5. It is recommended to run a standard curve with each group of assays, preferably in duplicate. Duplicate or triplicate unknowns are recommended.

## References

1. Burton, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of DNA. *Biochem. J.* **62**, 315–323.

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